Bioinspired interface for nanobiodevices based on phospholipid polymer chemistry

Kazuhiko Ishihara1,2,3,4,* and Madoka Takai1,3

1Department of Materials Engineering, 2Department of Bioengineering, and 3Center for NanoBio Integration (CNBI), University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
4Core Research Evolutional Science and Technology (CREST), Japan Science and Technology Agency, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

This review paper describes novel biointerfaces for nanobiodevices. Biocompatible and non-biofouling surfaces are designed largely based on cell membrane structure, and the preparation and functioning of the bioinspired interface are evaluated and compared between living and artificial systems. A molecular assembly of polymers with a phospholipid polar group has been developed as the platform of the interface. At the surface, protein adsorption is effectively reduced and the subsequent bioreactions are suppressed. Through this platform, biomolecules with a high affinity to the specific molecules are introduced under mild conditions. The activity of the biomolecules is retained even after immobilization. This bioinspired interface is adapted to construct bionanodevices, that is, microfluidic chips and nanoparticles for capturing target molecules and cells. The interface functions well and has a very high efficiency for biorecognition. This bioinspired interface is a promising universal platform that integrates various fields of science and has useful applications.

Keywords: bioinspired interfaces; phospholipid polymers; non-biofouling; molecular recognition; microfluidic devices; nanoparticles

1. INTRODUCTION

The cell membrane is a sophisticated, nanostructured molecular assembly in living organisms. It is mainly composed of phospholipid molecules, which play an important role in separating the intracellular cytoplasm from the external environment, and glycoproteins as receptor and membrane penetrate proteins are also faced on the surface (figure 1; Singer & Nicolson 1972). The phospholipids have hydrophobic alkyl chains and a hydrophilic polar group, and they are spontaneously assembled as a continuous membrane in an aqueous medium. The molecular assembly shows unique characteristics not only in biological aspects but also in terms of physicochemical functions. The favourable characteristics of the cell membrane are as follows: (i) it has the mechanical strength to sustain the cell morphology, (ii) it has the ability to maintain the concentration of specific chemicals in the cytoplasm, and (iii) it can act as a scaffold for functional membranes with proteins and glycoproteins. In living organisms, the cell membrane is also used as an important communication interface between cell-cell junctions. Higher ordered assemblies at the nanoscale would be excellent for revealing several kinds of functions. The cell membrane structure is the most attractive candidate for the fabrication of nanostructured biomaterials, which involves bio-, nano- and information technologies. One of the major phospholipid polar groups on the cell membrane is phosphorylcholine, which is an electrically neutral, zwitter-ionic head group. In biomimetic chemistry, phospholipid molecules have been used in the preparation of cell membrane-like structures, namely, liposomes and Langmuir–Blodgett membranes. However, the major disadvantage of the molecular assembly is its chemical and/or physical stability. Stabilization of the phospholipid assembly is a topic of interest in the construction of interfaces between living and artificial systems. This review summarizes bioinspired interfaces based on the characteristics of the cell membrane for biomedical devices and nanobiodevices, with attention to phospholipid polymers.

2. CELL MEMBRANE-INSPIRED SURFACE FOR BIOMEDICAL APPLICATIONS

2.1. Phospholipid polymers as the platform of bioinspired interfaces

2.1.1. Phospholipid assembly on the surface. Many studies have focused on the preparation of phospholipid-assembled surfaces that suppress many biological
responses (Ishihara 2000; Lewis 2000). As shown in figure 2, various types of phosphorylcholine-assembled layers on solid surfaces have been explored, and the ‘non-biofouling’ properties of these surfaces have been reported. Bonte et al. (1987) and Ringsdorf & Schlarb (1988) investigated the polymerization of phospholipids with a polymerizable group. They found that polymerized liposomes did not induce platelet aggregation in plasma or blood. Hence, the adsorption of liposomes on the polymer support was a good method for preparing a phospholipid-assembled surface. They were, however, unable to prepare a biomimetic membrane, because the polymerization ability and mobility of the phospholipid polymer were quite poor. Chapman et al. also studied blood-compatible surfaces from a biological perspective. Phospholipid molecules containing diacetylenic groups in their acyl chain were synthesized and polymerized upon irradiation with ultraviolet light (Chapman 1993). Polymerization of the diacetylene group results in a perfectly regular polymer, with a stable polymeric phospholipid. This should render the surface more biocompatible, especially when the polymer is engineered to mimic the host cell surface. Diacetylenic phospholipid can be coated on the surfaces of a variety of materials to emulate the cell membrane surface. Kono et al. (1989) reported that polyamide microcapsules treated with phosphatidylcholines suppressed platelet adhesion. Hall et al. (1989) performed a thromboelastographic study of a variety of surfaces treated with phosphatidylcholines and observed prolongation of clotting time when compared with untreated surfaces. Durrani et al. (1986) and Hayward et al. (1986) synthesized phosphorylcholine derivatives with dimethylsilyl chloride and ethanolamine groups. These phosphorylcholine derivatives were coated on glass or a variety of polymer surfaces by covalent bonding. Tegoulia & Cooper (2000) synthesized alkanethiols by using a variety of functional groups that reacted with a gold surface. It was clear that the alkanethiols fixed on the gold surface created a self-assembled monolayer (SAM). The water contact angle of the SAM surface prepared with alkanethiol having a hydrophilic polar group was less than that of the gold surface. On the SAM surface prepared with the phosphorylcholine group, neutrophil adhesion was effectively reduced. Marra et al. (1997) synthesized the phospholipid monomer 1-palmitoyl-2-(12-(acryloyloxy)dodecanoyl)-sn-glycero-3-phosphorylcholine as unilamellar vesicles and fused it onto alkylated glass. Free radical polymerization was performed in an aqueous solution. X-ray photoelectron spectroscopic analysis validated that the phospholipid
assembly had a close-packed monolayer formation. This formation is very stable under static conditions in water and air and in an environment with a high shear flow. Blood compatibility was assessed in a baboon arteriovenous shunt model, which revealed minimal platelet deposition over observation for 2 hours. Kohler et al. (1996) prepared a glass surface that reacted with 3-aminopropyltrimethoxysilane (APTMS).

Carboxylated phosphatidylcholine derivatives were coated with a coupling agent. Modification of a glass surface with the carboxylated phosphatidylcholine derivatives suppressed the number of adherent platelets. As an improvement over the two-step carboxylated phospholipid derivative reaction with APTMS, Lu et al. (2001) described an APTMS-functional phospholipid dimer that was used to coat surfaces and reduce protein adsorption.

Phospholipid-endcapped polymers were prepared and used for the modification of substrates, e.g. oligo(N,N-dimethylacrylamide) and a block co-oligomer with oligo(styrene) prepared using a photoiniferter-based quasi-living polymerization technique (Matsuda et al. 2003). The oligomer has amphiphilic properties and chemisorbs on a gold surface with hydrophobic anchoring. The surface coated with the oligomers reduced plasma protein adsorption and cell adhesion. Nederberg et al. (2004) reported a phospholipid monolayer-endcapped biodegradable polymer based on aliphatic polyester. This polymer formed a phospholipid monolayer group microdomain in the polyester matrix. The concentration of phospholipid monolayers located at the surface when the polymer was immersed in aqueous medium reduced protein adsorption.

2.1.2. Phospholipid polymers and structural regulation. For constructing blood-compatible polymer materials having a good stability, processability and applicability, a new concept involving a methacrylate monomer with a phospholipid monomer group, 2-methacryloyloxyethyl phospholipid (MPC), was proposed (Ishihara et al. 1996a). The synthesis of MPC was difficult; however, in 1987, Ishihara developed the synthesis route and a method for purifying MPC, using which a sufficient quantity of MPC of excellent purity could be obtained. Presently, a Japanese company produces this polymer on an industrial scale and is a worldwide provider of MPC. Thus, preparation of MPC with various other alkyl methacrylates or styrene became possible, and their blood compatibility was carefully evaluated (Ueda et al. 1992). The introduction of other monomer units could alter the property of the polymer (Lewis 2004). MPC can polymerize with other vinyl compounds through a conventional radical polymerization. This implies that it is very easy to design the structure of MPC to adapt to a modified substrate. Controlling the monomer ratio determines the MPC unit composition in the polymer. MPC polymers with other polymer architectures such as block-type copolymers and graft-type copolymers were also prepared by a conventional radical polymerization technique (Ishihara et al. 1994; Inoue et al. 2004).

Recent advancements in radical polymerization have yielded well-defined polymers—living radical polymerization of MPC has been reported and small molecular weight-dispersion polymers were obtained. The atom transfer radical polymerization (ATRP) of MPC has been investigated, and then block-type copolymers with poly(MPC) segment were obtained (Lobb et al. 2001; Ma et al. 2002, 2003). Also, as another living radical polymerization mechanism, reversible addition fragmentation transfer polymerization was applied to obtain well-controlled MPC polymers (Yusa et al. 2005; Inoue et al. 2005; Seo et al. 2008). Surface-initiated living radical polymerization has been hypothesized to have potential in synthesizing a brush-type polymer graft layer (Iwata et al. 2004; Feng et al. 2005; Hoshi et al. 2008). The functional MPC polymer is useful for surface modification and can convert block-type and graft-type copolymers (figure 3).

2.1.3. Fundamental property of phospholipid polymers. Excellent anti-thrombogenic properties were observed when the MPC polymers came in contact with platelet-rich plasma (Ishihara et al. 1990b; Iwasaki et al. 2001) or human whole blood even in the absence of an anticoagulant (Ishihara et al. 1992). Figure 4 shows the scanning electron microscopy (SEM) images of the polymer surfaces after contact with human whole blood. On the surface of poly(n-butyl methacrylate) (PBMA), a fibrin net completely covered the bead surfaces and many blood cells adhered. On the other hand, no fibrin deposition and cell adhesion could be found on the poly(MPC-co-BMA) (PMB; figure 3) surface. These results clearly show that MPC polymers have excellent anti-thrombogenic properties and MPC moieties in the polymer are an important element in the anti-thrombogenicity of the polymers. Protein adsorption is one of the most important phenomena in determining the biocompatibility of materials. In general, proteins adsorb on a surface within a few minutes of the material coming in contact with body fluids such as blood, plasma or tears. Protein adsorption on the MPC polymers from human plasma determined by radioimmunoassay and an immunogold-colloid labelling technique showed that the amount of adsorbed protein was quite small and decreased with an increase in the MPC moiety (Ishihara et al. 1991). Figure 5 shows the result of the radioimmunoassay used to detect proteins on the plasma-contacting surface after a 60 min contact with poly(BMA) and PMB. Not only major components of plasma proteins such as albumin (Alb), fibrinogen (Fib) and γ-globulin (IgG), but also minor components were observed on the surface of every material. Protein adsorption was reduced with an increase in the MPC unit composition. In the case of PMB with 30 mol% of the MPC unit, the concentration of every adsorbed protein was reduced drastically compared with that on poly(BMA).

The equilibrium amount of the proteins Alb and Fib adsorbed on the polymer surface is measured and represented with the free water fraction in the hydrated polymers as shown in figure 6 (Ishihara et al. 1998).
Poly(2-hydroxyethyl methacrylate) (HEMA), poly(acrylamide (AAm)-co-BMA) and poly(N-vinylpyrrolidone (VPy)-co-BMA) showed greater protein adsorption than the MPC polymers. The increase in the MPC mole fraction was effective in reducing the amount of protein adsorption. It was reported that the theoretical amounts of Alb and Fib adsorbed on the surface in a monolayer state are 0.9 and 1.7 mg cm$^{-2}$, respectively. On the surface of the MPC polymers, the amount of adsorbed proteins was less than these theoretical values. This implies that the proteins that were attached to the surface could be very easily detached by rinsing. Thus, it is considered that the phosphorylcholine group can reduce protein adsorption effectively.

A previous study suggested that polymers having a hydroxyl group, such as poly(HEMA), could incorporate water molecules at the surface and form a network structure of water molecules (Tsuruta 1996). Protein adsorption starts with protein trapping by this network structure. The longer the contact of a protein with the surface, the greater is the likelihood of the protein interacting with the surface, undergoing a conformational change, and inducing irreversible adsorption. This is a very acceptable consideration to explain the difference in protein adsorption behaviour between the MPC polymers and other amphiphilic polymers, including poly(HEMA). Most of the polymeric materials have a negative surface charge even if they do not have any negatively charged groups. In fact, surface $\xi$-potentials of poly(ethylene terephthalate) (PET) and poly(BMA) are $-41$ and $-36$ mV, respectively (Ishihara et al. 1998). The hydrated polymer poly(HEMA) also has a negative $\xi$-potential of $-16$ mV. On the other hand, when the PET surface was coated with PMB, the $\xi$-potential became 0 ($-0.4$ mV). This characteristic is important for understanding the surface water structure of these polymers. On PET, poly(BMA) and poly(HEMA) surfaces, water molecules bind via electrostatic interactions (dipole-dipole interactions). PMB effectively prevents the interaction between the surface and water molecules. Regarding the structure of water molecules in the polymer aqueous solution or that at the polymer surfaces, Kitano et al. (2000, 2003) mentioned hydrogen bonding among water molecules with vibrational spectroscopy. They demonstrated that the
effective biomedical materials, and it could provide materials to develop new blood-contacting artificial organs. Indeed, MPC polymers are in use as coating materials for various medical devices, including cardiovascular stents (Lewis et al. 2001a; Lewis & Stratford 2002), oxygenators (Myers et al. 2003) and catheters (Lewis et al. 2001b; Gobeil et al. 2002). In the case of stents, the metal surface is covered with self-curable MPC polymer to prevent initial thrombus formation when it is implanted into a blood vessel. The surface inhibits blood coagulation during the first stage, and the stent is then embedded into the blood vessel wall. Kihara et al. developed an implantable artificial blood pump composed of titanium alloy (Kihara et al. 2003). The surface of this pump was modified with high-molecular-weight PMB with 30 mol% of MPC units. It could attach to the surface physically; a stable coating layer is produced by a simple dip coating method by using its ethanol solution. The performance of the PMB layer for preventing thrombus formation was good and was in continuous use for more than 823 days in animal tests without any anticoagulant treatment. In 2005, the artificial blood pumps were implanted in human subjects under a clinical trial, and they have shown good results continuously to date.

2.2. Protein and polysaccharide binding for molecular recognition

Immobilization of biomolecules, including proteins, polysaccharides and DNA, is important to prepare sensors, monitors and diagnostic devices based on bioaffinity. To that end, an easy method is necessary for immobilization under mild conditions. Additionally, the activity of biomolecules after immobilization should be maintained as that in the native state. Thus, Ishihara et al. designed and synthesized a novel phospholipid polymer based on the chemical structure of PMB: poly(MPC-co-BMA-co-p-nitrophenyloxycarbonyl poly(ethylene glycol) methacrylate (MEONP)) (PMBN; figure 7; Konno et al. 2004; Park et al. 2004; Sakai-Kato et al. 2004; Takei et al. 2004). One of the monomer units, the MEONP unit, has an active ester group in the side

Figure 5. Adsorption of plasma proteins on surfaces. CPM, count per minute; Alb, albumin; Fib, fibrinogen; IgG, immunoglobulin G; C5, coagulation factor XII; VIII, coagulation factor VIII; HMWK, high-molecular-weight kininogen; FN, fibronectin; PBS, phosphate-buffered saline.

Figure 6. Relationship between the free water fraction of the polymer and the amount of adsorbed protein on the polymer surface. Circles, Alb; squares, Fib.

The phospholipid polymer having a phosphorylcholine group, such as the MPC polymers, is one of the water-soluble MPC polymers did not disturb the hydrogen-bond formation among water molecules. Also, the vibration spectra of water molecules at the MPC polymer surface were almost the same as that of pure liquid water. This means a small number of binding water molecules are at the surface of MPC polymers. Kobayashi et al. investigated the poly(MPC) brush surface and observed super-hydrophilic properties at the surface (Kobayashi et al. 2007). The surface free energy of the poly(MPC) brush surface was estimated to be 73 mN m\(^{-1}\), which is quite similar to that of water. These results clearly indicated that the water structure is maintained as free water in the MPC polymer systems.

The circular dichroism (CD) spectrum of a protein adsorbed on a polymer surface provides useful information about the conformational change during adsorption (Ishihara et al. 1998; Kondo et al. 1993). From the CD spectra of proteins adsorbed on polymer surfaces, the secondary structure of the adsorbed proteins was calculated to determine the \(\alpha\)-helix content. The \(\alpha\)-helix contents of Alb and Fib, which are assumed to be ‘native’ secondary structures, were 54 and 19 per cent, respectively. When these proteins adsorbed on the polymer surface, changes in the \(\alpha\)-helix content were observed. On the surface of the MPC polymers, the \(\alpha\)-helix content of both adsorbed Alb and Fib was almost at the same level as that of the native proteins. The proteins adsorbed on the MPC polymers could maintain their original higher \(\alpha\)-helix level compared with those on poly(HEMA). It is concluded that when the free water fraction on the polymer surface is maintained at a higher level, the proteins can contact the surface reversibly without a significant conformational change. The free water fraction must be one of the important factors to be considered for the biocompatibility and non-biofouling property of polymeric materials.

The phospholipid polymer having a phosphorylcholine group, such as the MPC polymers, is one of the
This PMBN was coated on a polymer substrate and an enzyme protein was immobilized to capture a specific substrate. The enzymatic reaction proceeded well, and the product could be detected. The polymer nanoparticles were coated with PMBN for immobilization of the antibody and enzyme. Poly(l-lactic acid) (PLA) was used as a core substance where the surface was covered with PMBN chains (PMBN/PLA nanoparticles). Watanabe and Ishihara have successfully performed sequential enzymatic reactions via three types of enzymes (Watanabe & Ishihara 2006, 2008a,b): acetylcholine esterase; choline oxidase; and horseradish peroxidase-labelled IgG. These enzymes were immobilized onto PMBN/PLA nanoparticles. The order of enzymatic activity was believed to gradually increase. It is considered that enhancement of the reactivity was expected, and a large amount of the degradation product would then be recovered. The sequential enzymatic reactions proceeded effectively relative to those of the enzyme solution, since a higher local concentration of each enzyme and effective diffusion of the newly produced substrates were observed.

Non-specific adsorption of proteins on the polymer nanoparticles was examined using PMBN/PLA nanoparticles and conventional polystyrene (PS) nanoparticles (Goto et al. 2008a). Numerous Alb molecules were adsorbed on the commercially available PS nanoparticles, whereas adsorption was barely noted on the PMBN/PLA nanoparticles after reaction with glycine to avoid a reaction between MEONP units on the nanoparticles and Alb. The amount of Alb adsorbed on the PMBN/PLA nanoparticles was approximately 1/300 when compared with that on the PS nanoparticles. That is, as a platform, the PMBN/PLA nanoparticles showed excellent suppression of non-specific protein adsorption.

To recognize the activity of biomolecules after immobilization, the dissociation constant of the antigen/antibody complex on the PMBN/PLA nanoparticles was measured. The dissociation constant ($K_d$) could be calculated from these plots, and it was observed to be $2.7 \times 10^{-7}$ M for the anti-Alb antibody-immobilized PMBN/PLA nanoparticles (anti-Alb PMBN/PLA nanoparticles) and $1.3 \times 10^{-8}$ M for the anti-Alb antibody-immobilized PS nanoparticles modified with a succinimide moiety (anti-Alb PS nanoparticles) on the surface. Thus, the affinity of the anti-Alb antibody to Alb observed on the anti-Alb PMBN/PLA nanoparticles was approximately 200-fold higher than that on the anti-Alb PS nanoparticles. The $K_d$ value generally ranges from $10^{-7}$ to $10^{-10}$ M for an antigen–antibody complex. The $K_d$ value of the anti-Alb antibody immobilized on the PMBN/PLA nanoparticles for Alb is considered valid, while that for the anti-Alb antibody immobilized on the PS nanoparticles is higher than the reported value. This indicates that the antibody immobilized on the PMBN/PLA nanoparticles had a strong affinity towards the antigen, maintaining the activity of the antibody even when immobilized on the nanoparticles. These results indicate the effects of the phosphorylcholine groups in preventing the denaturation of the antibody.

Kinoshita et al. (2007) have reported the immobilization of DNA moieties. They focus on the hybridization properties with regard to a suitable surface chemistry for a cyclic olefin copolymer (COC) surface with PMBN and discuss new approaches for the application of an on-chip DNA detection method through multiple primer extension (MPEX) by DNA polymerase (figure 8). DNA templates hybridize to solid surface-bound primers, which are then elongated with DNA polymerase to produce a copy of the hybridized template in the 5’ to 3’ direction, and deoxynucleotidyl triphosphates (dNTP) as extender units are incorporated into their nucleic acid chain elongation products. Also, a DNA amplification procedure similar to the polymerase chain reaction (PCR) method on the solid surface occurs via the following mechanism. At each cycle, hybridization between the DNA primers and the DNA templates present in the solution participates in the primer elongation process before being released back into the system, which can react with a specific biomolecule via condensation with an amino group of the biomolecules.

The dissociation constant of the antigen/antibody complex on the PMBN/PLA nanoparticles was measured. The dissociation constant ($K_d$) could be calculated from these plots, and it was observed to be $2.7 \times 10^{-7}$ M for the anti-Alb antibody-immobilized PMBN/PLA nanoparticles (anti-Alb PMBN/PLA nanoparticles) and $1.3 \times 10^{-8}$ M for the anti-Alb antibody-immobilized PS nanoparticles modified with a succinimide moiety (anti-Alb PS nanoparticles) on the surface. Thus, the affinity of the anti-Alb antibody to Alb observed on the anti-Alb PMBN/PLA nanoparticles is higher than the reported value. This indicates that the antibody immobilized on the PMBN/PLA nanoparticles had a strong affinity towards the antigen, maintaining the activity of the antibody even when immobilized on the nanoparticles. These results indicate the effects of the phosphorylcholine groups in preventing the denaturation of the antibody.

**Figure 7. Molecular design of the MPC polymer for bioconjugation (PMBN).**

![Molecular design of the MPC polymer for bioconjugation (PMBN).](image-url)
solution phase after denaturation at 95°C. The DNA templates repeatedly hybridize to attach neighbouring primers to the solid surface and form additional fluorescent copies. DNA oligonucleotide probes are covalently immobilized on the PMBN surface via amine at their 5' terminus. This type of surface chemistry offers extraordinarily stable thermal properties because of the absence of a pre-activated glass slide surface. These results suggest that the plastic platform PMBN can clear challenging technical hurdles for DNA microarrays in hybridization-based analysis in the near future. In addition, when the oligonucleotide DNA template is in the DNA array solution, the sequence-specific primer extension reaction and sequential DNA amplification can be processed on the solid surface by thermal cycling, as used in the case of PCR. In this case, oligonucleotide DNA primers on the PMBN can be highly amplified exclusively at the picomolar concentration range of complementary oligonucleotide templates. Finally, it was suggested that these completely different approaches to genome analysis, such as single nucleotide polymorphisms analysis, sequencing-by-synthesis via MPEX biosynthesis and the detection of non-coding micro RNA by reverse transcriptase could be developed using their DNA microarray platform by PMBN through MPEX techniques.

Iwasaki et al. (2007) immobilized carbohydrate side chains on PMB for obtaining specific interaction towards cells. They prepared polycarbohydrate-immobilized MPC polymers on PMBN for obtaining specific interaction towards cells. They prepared poly(MPC-co-BMA-co-2-lactobionamidoethyl methacrylate (LAMA)) (PMBL) and coated it on substrates by solvent evaporation. Cells of the human hepatocellular liver carcinoma cell line (HepG2) having asialoglycoprotein receptors (ASGPRs) were seeded on the polymer surfaces. On poly(BMA), many adherent cells were observed and were well spread with monolayer adhesion, but cell adhesion was reduced on PMB. HepG2 adhesion was observed on PMBL because the cell has ASGPRs; the number of cells adhering to the PMBL polymer surfaces increased with the density of the galactose residues on the surface. By contrast, adhesion of NIH 3T3 cells to PMBL was reduced in a manner similar to that on PMB, because the NIH 3T3 cells did not have ASGPRs. Cell adhesion to the PMBL surface was well regulated by ligand–receptor interactions. Furthermore, some of the cells adhering to the PMBL surface had a spheroid form, and similarly shaped spheroids were scattered on the surface. Although poly(BMA-co-LAMA) (PBL) has galactose residues, the adherent cells were spread in a manner similar to those on poly(BMA). The MPC units in PMBL contribute to the spheroid shape formation of HepG2 cells. The amount of Alb secreted from a cell was compared with the chemical structure of the substrate. The spheroid cells cultured on the PMBL surface secreted much more albumin than the spreading cells that adhered to poly(BMA). They concluded that the carbohydrate-immobilized MPC polymers produced a suitable interface for biorecognition and preservation of cell function.

3. NANOBIODEVICES FOR DIAGNOSIS

3.1. Microfluidic devices for highly sensitive diagnosis

3.1.1. Preparation of a nanostructured surface by electrospray deposition. Nishizawa et al. (2008) developed a new solid biointerface by integrating an MPC polymer (PMBN) with a nanoscale surface modification process known as electrospray deposition (ESD). In the enzyme immunoassay using this surface, the sensitivity was significantly enhanced. The surface prepared by ESD has a nanosphere-shaped structure, and therefore the highly uneven surface causes a drastic increase in specific signals owing to an increase in the surface area. The PMBN surface, which can conjugate antibodies, prevented the non-specific protein adsorption. The stability of the antibodies immobilized on the PMBN surface was improved drastically. The nanosphere-shaped PMBN surface can be used to yield a highly sensitive, stable and reliable assay. Figure 9 shows the surface image of an Au/polymide sheet dip coated with an ethanol solution of PMBN and the sheet sprayed with a 5 wt% ethanol solution of PMBN by the ESD device at a voltage of 30 kV. A surface dip coated with PMBN is very smooth, while the nanosphere-shaped polymer network observed on the surface sprayed by ESD is highly uneven; this surface contributes to an increase in the surface area, which is believed to cause a drastic increase in specific signals.

3.1.2. Immobilization of antibody on the EDS surface. In order to confirm the stability of the antibodies immobilized on these PMBN surfaces, they were incubated under dry conditions at 37°C for several days, and the assay was then carried out as an accelerated test. The surfaces coated with Alb as a blocking reagent and the uncoated surface exhibited a low stability of the immobilized antibodies. Their residual activities decreased drastically in 5 days. This indicates that the physical adsorption of the antibody on the PS microtitre plate easily induces denaturation of the antibody. When compared with the

![Figure 8. Schematic of the MPEX technique using immobilized oligoDNA primer.](http://rsif.royalsocietypublishing.org/Downloaded from http://rsif.royalsocietypublishing.org/)}
Alb-blocked surface, the PMBN-coated surface had maintained 50 per cent of the residual activity even after 15 days. This indicates that the PMBN surface provides a stable condition for the antibodies owing to the presence of the MPC unit and the chemical binding to the polymer via the oxyethylene chain. The reduction in the denaturation of the immobilized antibodies on the PMBN surface results in a highly specific signal and realizes a high signal-to-noise ratio. Additionally, long-term stability of immobilized antibodies is required for heterogeneous immunoassays used for diagnosis. A commercial enzyme-linked immunosorbent assay (ELISA) kit is freeze-dried after primary antibody immobilization on the plate, and the kit is used after storage in a refrigerator. The denaturation of the immobilized primary antibodies on the stored plate is a significant problem associated with the reliability of the assay. Therefore, the PMBN surface offers great advantages in terms of the practical use of the immunoassay.

To evaluate the increase in the surface area achieved by the ESD method, ELISA was carried out for these surfaces (figure 10). With regard to specific signals, the PMBN surface prepared by dip coating had a very low absorbance, whereas the absorbance of the ESD-sprayed surface was considerably higher—it was twice of that in the case of Alb blocking. This indicates that a large amount of antibodies was immobilized on the nanosphere-shaped surface because of the increase in the surface area. In the assay using the PMBN surface sprayed by ESD, the signal/background (S/B) ratio was significantly enhanced to 15. The main factors responsible for this were the enhancement of specific signals and the reduction of background signals, which occur due to the non-specific binding of the analyte or enzyme.

3.1.3. ELISA in microfluidic devices. ELISA using a microfluidic chip with PMBN-coated surfaces was carried out to evaluate the specific and non-specific signals (Nishizawa et al. 2006). The microfluidic chip was synthesized from polydimethylsiloxane (PDMS). The depth of the microchannel was 100 μm, and the width was 300 μm and 2 mm for the channel and reaction area, respectively. Au was patterned on slide glass as the electrode for EDS and PMBN was then sprayed by ESD on the 2 mm × 2 cm Au area. Finally, the microfluidics on PDMS and the slide glass were fabricated to create a microfluidic chip.

In the case of a non-coated Au surface using Alb as a blocking reagent, specific signals (human thyroid stimulating hormone, TSH = 10 μIU ml⁻¹ (signal); open bars, [TSH] = 0 μIU ml⁻¹ (background). The asterisks indicate p < 0.01.
The PMBN surface sprayed by ESD showed highly specific signals because of a large number of conjugated antibodies on increasing the surface area. Additionally, the background level was low because the MPC units reduce non-specific protein adsorption on the microchip. Furthermore, the assay time on the microchip was shortened to 20 min from 4 hours. That is, the amount of sample needed was only 5 μl by using microfluidic ELISA chips, whereas it is 2 ml in a conventional system. The efficacy of analysis may be evaluated by both the time and amount of sample consumed for analysis; here, it was dramatically improved: a value as high as 4800 times was noted by using the microfluidic ELISA chip with the bioinspired interface.

3.2. Nanoparticles for bioimaging

3.2.1. Functioning of the core matrix of nanoparticles coated with PMBN. It has already been described that the polymer nanoparticles coated with PMBN are useful for capturing a specific compound by the immobilization of biomolecules. In this case, the core of the nanoparticles is one of the hydrophobic polymers, such as PLA and PS. To use the core part of the nanoparticles, hybridization with magnetic nanoparticles, semiconductor nanocrystallites or organic nanopowdered compounds, including drugs, may be applied (figure 12). Goto et al. (2008b) used a core with semiconductor nanocrystallite quantum dots (QDs). QDs have gained much interest as a promising alternative to organic dyes for biological imaging. Those ranging in size between 2 and 6 nm have unique optical properties: material- and size-dependent emission spectra, a wide absorption spectrum, high quantum yields, simultaneous multicolor emissions and particularly excellent resistance to photobleaching. This photostability is a critical feature in most fluorescence applications, particularly for the long-term monitoring of labelled substances, and is an area in which QDs have a singular advantage over organic dyes. As QDs themselves are hydrophobic owing to stabilizing detergents, they are hardly dispersed in an aqueous medium alone. Thus, the key to developing
QDs as a tool in biological systems is to achieve good dispersion ability in an aqueous medium and compatibility with biological components, including cells.

The fabrication of polymer nanoparticles, which contained QDs (PMBN/PLA/QD) and were coated with PMBN, was carried out by a solvent evaporation technique. The PMBN/PLA/QD thus obtained has good dispersion ability in water and phosphate-buffered saline (PBS, pH 7.4), and retains high fluorescence. The optical properties were very similar to those of uncoated commercial QDs in toluene. The encapsulation of QDs provided extended stability, and no change in the dispersion ability or fluorescence was observed over a six month period (stored at 4°C), and the PMBN/PLA/QD is stable between pH 4.0 and pH 9.0. Overall, the encapsulation of QDs by using PMBN and PLA did not have any influence on the optical properties of the QDs.

3.2.2. Analysis of the cell material interaction based on bioimaging.

The protective ability of PMBN/PLA/QD against non-selective cellular uptake was assessed using HeLa cells in an in vitro system (Goto et al. 2008b). To avoid chemical reactions between active ester groups in the MEONP units and proteins in the culture medium, glycine, a simple amino acid, was immobilized to active ester groups on the surface of the PMBN/PLA/QD (glycine–PMBN/PLA/QD). A fluorescence image of the HeLa cells incubated with glycine–PMBN/PLA/QD is shown in figure 13. However, we could not observe any fluorescence from the cells. The internalization of QDs provided extended stability, and no change in the dispersion ability or fluorescence was observed over a six month period (stored at 4°C), and the PMBN/PLA/QD is stable between pH 4.0 and pH 9.0. Overall, the encapsulation of QDs by using PMBN and PLA did not have any influence on the optical properties of the QDs.

Figure 13. Cellular uptake of polymer nanoparticles by specific biomolecules on the surface. (a, c) Phase contrast microscopy view and (b, d) fluorescence microscopy view.

R8–PMBN/PLA/QD

glycine–PMBN/PLA/QD

4. FUTURE PERSPECTIVE

In this review, recent developments in bioinspired interfaces constructed with phosphorylcholine groups have been introduced. It particularly focuses on non-specific and specific interactive bioconjugate interfaces for the establishment of a universal platform in the biomedical field. The structure of the cell membrane is one of the excellent examples to realize interface design. The essential factor in these developments is simultaneous multiple achievements: suppression of protein adsorption, easy fabrication, immobilization of biomolecules, maintenance of higher biofunctions and high capturing ability of the target molecules. The phospholipid polymer composed of the MPC units provides a considerable number of advantages for constructing the bioinspired interface. MPC facilitates versatile polymerization methods with comonomers, and it can suppress non-specific protein adsorption, higher hydration property and refolding property of the biomolecules. Controlling interactions with cells is of considerable importance in biomedical fields, including nanobioengineering and
cell and tissue engineering. The bioinspired interfaces described here are a promising design for realizing a universal platform that integrates polymer chemistry, material science and engineering, biochemistry, cell biology and nanofabrication.

The authors extend their appreciation to Prof. Tadashi Kokubo, Chubu University, for providing the opportunity to contribute this review paper in a special issue of this journal and to Prof. Yasuhiko Iwasaki, Kansai University, and Prof. Junji Watanabe, Osaka University, for their help with useful information in this research field. Additionally, the authors thank Dr Tomohiro Konno and Dr Ryosuke Matsuno, University of Tokyo, for helping in the preparation of this manuscript.

REFERENCES


Gobeil, F., Juneau, C. & Plante, S. 2002 Thrombus formation on phospholipid (doe:10.1016/j.biomaterials.2002.06.102)


Kokubo, Chubu University, for providing the opportunity to contribute this review paper in a special issue of this journal.
Kitano, H., Imai, M., Mori, T., Gemmei-Ide, M., Yokoyama, Y. & Ishihara, K. 2003 Structure of water in the vicinity of phospholipid analog copolymers as studied by vibrational spectroscopy. 

Langmuir 19, 10 260–10 266. (doi:10.1021/la0349673)


